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Characterization of Automotive Emissions by Bacterial Mutagenesis Bioassay: A Review

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16. ABSTRACT

Due to the growing numbers of diesel passenger autobiles in the United States, there has been an expanded effort to understand the health effects of airborne pollutants arising from increased automotive emissions. Bacterial mutagenicity testing has played an important role in the characterization of genotoxic effects and components arising from these combustion products. This review examines published material concerning the bacterial mutagenicity of automotive emissions. In addition, the paper explores factors that modify the mutagenicity of mobile-source emissions, the use of bacterial tests for the comparison of various mobile source emissions, and the use of bacterial tests to examine the phenomena of mammalian uptake and metabolism.

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Characterization of Automotive Emissions by Bacterial Mutagenesis Bioassay: A Review

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Due to the growing numbers of diesel passenger automobiles in the United States, there has been an expanded effort to understand the health effects of airborne pollutants arising from increased automotive emissions. Bacterial mutagenicity testing has played an important role in the characterization of genotoxic effects and components arising from these combustion products. This review examines published material concerning the bacterial mutagenicity of automotive emissions. In addition, the paper explores factors that modify the mutagenicity of mobile-source emissions, the use of bacterial tests for the comparison of various mobile source emissions, and the use of bacterial tests to examine the phenomena of mammalian uptake and metabolism.

Key words: diesel, gasoline, Ames test, Salmonella, fuel, combustion

INTRODUCTION

The United States has approximately 130 million passenger cars and light-duty trucks, nearly one light-duty vehicle registered for each adult. The sales and servicing of automobiles and trucks account for about 25% of the US retail market [Gray and von Hippel, 1981]. In 1980 this fleet of vehicles consumed approximately 2 billion barrels of oil. Since engineering tests have shown a 25% or greater improvement in fuel economy in light-duty vehicles equipped with diesel engines versus those equipped with gasoline engines, diesel vehicle sales are expected to increase from 4% (1980) to 15% (1985) of the new car market. This "dieselization" has sparked new interest in the health effects of mobile-source emissions. Although earlier work [Kotin et al, 1954, 1955] had demonstrated that diesel and gasoline emissions have potential carcinogenic activity, it was not until 1978 that industry and government expanded their efforts toward understanding whether or not mobile-source emissions could

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show any potential genotoxic health effects. This issue received priority after Huisingh et al [1978] and a cautionary notice for laboratory workers issued by the US Environmental Protection Agency (EPA) [Gage, 1977] reported that organic fractions from the exhaust particles of diesel vehicles were mutagenic in the Ames Salmonella typhimurium plate incorporation assay [Ames et al, 1975]. The purpose of this review is to recognize and document the role that bacterial mutation tests have played in characterizing mobile source emissions for genotoxic activity. A summary of this review was given at the EPA 1981 Diesel Emissions Symposium held October 5–7, 1981, in Raleigh, NC [Claxton, 1981b].

Although the original report by Huisingh et al [1978] was quite extensive—examining, for example, chemical and physical properties of fractions from exhaust organics, multiple vehicles, and multiple fuels—many questions remained unanswered. Researchers in government and industry, in the service of the public interest, have used bacterial mutagenicity tests to answer several critical questions concerning sample generation, collection, extraction, fractionation, bioassay, statistical significance, and relevance. For example, Wei et al [1980] postulated that ". . . controversies on the biological hazards of diesel emissions will remain unresolved until more is known about the chemical identities of the direct-acting mutagens." Fractionation directed by bacterial bioassay results enables investigators to follow the distribution of genotoxic activity among different chemical classes before compound identification is complete. In addition, microbial tests allow comparative measurements of genotoxic activity from roadside-exhaust, smog-chamber, and dilution-tunnel samples. The purpose of this paper, therefore, is to recognize and document the role that bacterial mutation tests have played in characterizing mobile-source emissions for genotoxic activity. The bacterial mutagenicity of mobile-source emissions is considered according to the following schema: (1) generalized observations; (2) generation of emissions; (3) collection of emissions samples; (4) extraction of particulate samples; (5) fractionation and identification of individual chemical components; (6) applicability and relevance of bacterial bioassays; and (7) data transformation and statistical analysis of research data.

GENERALIZED OBSERVATIONS

Huisingh et al [1978] and most other investigators have used the Salmonella typhimurium plate incorporation assay [Ames et al, 1975] as the primary test protocol. Although some investigators employ all five tester strains recommended by Ames for general screening, many investigators work exclusively with strains TA98 and TA100, primarily for two reasons: First, the sample amounts available have been relatively limited; and second, TA98 and TA100 have been the strains most responsive to the soluble organic fraction (SOF) extracted from mobile-source emission particles. Total extracts give negative results in tests with TA1535, which responds to base pair-substitution mutagens; however, since bioassay-directed chemical fractionation studies have not generally used this strain, any fractions containing mutagens that cause base pair substitution may be overlooked. Strain TA1538 provides even more interesting observations. TA1538 exhibits an increased response upon the addition of an Aroclor-induced liver homogenate, whereas TA98 exhibits the same or a decreased response under exogenous activation. The different responses of TA1538 and TA98 suggest either that (1) TA1538 (although not providing as many revertants per plate) distinguishes the presence of indirect-acting mutagens not readily detected by TA98;

or (2) TA98 detects frameshift promutagens that are S9-deactivated, which TA1538 is not capable of detecting. Researchers may thus be underestimating the importance of indirect-acting frameshift mutagens within mobile emissions.

Using the knowledge of mutagens available at that time, Wei et al [1980] surmised that nitro-substituted polycyclic aromatic hydrocarbons (PAH) were the probable mutagens associated with diesel exhaust emissions. Nitro derivatives of some PAHs have been identified in ambient air samples [Jäger, 1978; Wang et al, 1980; Talcott and Harger, 1981; Pitts et al, 1982b]. Rosenkranz et al [1980] and Mermelstein et al [1981] characterized nitro reductase-deficient bacterial strains that allowed for the initial recognition of nitroarenes in mobile emissions. Nitroarenes were identified in diesel exhaust through the use of these strains by Claxton [1981a], Claxton and Kohan [1981], and Löfroth [1981].

The 8-azaguanine mutation system has also shown positive results with various mobile source emissions. It can be used to give a more quantitative approach and identify a wider spectrum of compounds [Claxton and Kohan, 1981; Liber et al, 1980; Barfknecht et al, 1981b]. However, this system has not been generally employed.

The widely used liver homogenate systems generally reduce the mutagenic response of diesel organics (excepting TA1538). However, exogenous activation in gasoline exhaust organics produces an enhanced mutagenic response, a fact that has received little emphasis in the literature. Thus, diesel and gasoline vehicles are demonstrated to emit different mutagenic compounds.

FACTORS THAT MODIFY THE GENERATION OF MUTAGENS WITHIN MOBILE SOURCE EMISSIONS

In the generation of emissions from a combustion system, there are five fundamental components to consider: the fuel, the fuel's oxidant, the fuel's diluents, the type and degree of combustion, and the atmospheric and environmental conditions.

Fuels

Fuels could influence the mutagenicity of exhaust organics by either the direct contribution of mutagens or by supplying the precursors for mutagens created during the combustion process. The diesel fuel used by Huisingh et al [1978] was negative when tested directly in the Salmonella bioassay. Lebowitz et al [1979] also reported that diesel fuel was negative. The diesel fuel JP-4 and two types of gasoline were reported negative by Wang et al [1978a,b] when tested with TA98. Various crude oils and some of their distillates, however, were observed as positive in the Ames test [Brusick and Matheson, 1978a,b]. Positive results, for example, were reported for some natural, syncrude, and shale oil crudes (and some of their distillates) by Calkins et al [1980] and Calkins and Krahn [1979]. In each case, however, the naphtha distillate was negative. Epler et al [1978b] and Guerin et al [1980] demonstrated that coal-derived petroleum substitutes could provide a tenfold increase in bacterial mutagenicity over a similar natural product. They also reported that the petroleum crude activity was found primarily in the neutral fraction, while significant activity was found in both the neutral and basic fractions of derived fuels. For the neutral fraction, Guerin et al [1980] demonstrated that aromatic amines were the predominant mutagenic constituent.

In contrast to the above studies, when Henderson et al [1981] separated diesel fuel into an aromatic and an aliphatic fraction, they found both fractions mutagenic

using strain TA100. These varying reports may indicate vast differences between sources of diesel fuel and bioassay techniques employed. A further possible variable is the presence of minor components not detected unless fractionated components of the fuel are used for testing. In addition, Henderson et al [1982] noted that exposure to nitrogen dioxide (NO_2) dramatically increased the response of both fractions. This finding is supported by the work of Pitts [1979], who exposed an indirect-acting mutagen, benzo(a)pyrene, to NO_2 and generated a direct-acting derivative. Such a correlation was not unexpected, since some nitrous acid also could have been present during these exposure conditions. The precursor effect of the fuel appears to have been demonstrated by Huisingh et al [1978]. Upon testing the effects of seven different fuels in two different vehicles, they found a wide range of mutagenic activity in the emission organics. The results of McClellan [1980b] are similar, also suggesting that fuels high in aromatic content produce a more notable mutagenic response. Although results support the hypothesis that fuels mainly supply precursor material for the mutagens in exhaust emissions, more research would be needed to rule out any major concentrating effect.

The Fuel's Oxidant and Diluent

The oxidant for both spark-ignited (gasoline) engines and compression-ignited (diesel) engines is, of course, oxygen. Nitrogen, which composes approximately 78% of the atmosphere, is the most common diluent. Water vapor, other inert gases, and some inorganic ash are the other diluents present. At high temperature, some inert nitrogen enters into the combustion reaction, and nitrogen oxides are produced. In addition, excess oxygen, lubricating oils, and/or fuel behave as diluents. (For an introduction to combustion and emission chemistry, see the text by Edwards [1977].) As will be shown in a later section, nitrated and oxygenated components of incomplete combustion contribute to the mutagenicity of emission products. Crankcase oils have also been investigated for possible mutagenic activity. Wang et al [1978a], Hermann et al [1980], and Löfroth [1981] each reported that unused crankcase oils are nonmutagenic, but that used crankcase oils from gasoline engines give a positive response. In addition, Löfroth [1981] stated that (1) metabolic activation increased any mutagenic response seen; (2) the response increased with vehicle mileage; and (3) this positive response was not seen with used oil recovered from a diesel engine.

Type and Degree of Combustion

Within mobile sources, the type of combustion depends upon the type of power source that is used. In the United States, the most common power train for light-duty vehicles is the typical spark-ignited gasoline engine. Diesels, which are reciprocating-compression ignition engines, are most often the power source for heavy-duty trucks, buses, locomotives, and vessels. Other engines that have shown some utility or are undergoing further research are: gas turbine and Wankel internal-combustion, Rankins and Stirling cycle external-combustion, and electric.

The effect of type and degree of combustion on mutagenicity is examined by comparing the results from different power sources and/or vehicles. Although a few authors did not describe the engine or vehicle used in their research, most gave at least a limited description. Huisingh et al [1978] employed two heavy-duty engines and three light-duty engines. Although direct comparison of differing sources was not the primary purpose of that research, it provided a mobile source comparison

based on bacterial mutagenicity. Particle exhaust organics from heavy-duty engines were tested in TA98, TA1535, TA100, TA1537, and TA1538, both with and without exogenous activation. Both engines showed very similar qualitative results, with the four positive strains having decreasing activity in the order TA100 > TA98 > TA1538 > TA1537. Without activation, TA1535 was negative with samples from both engines; however, with activation, one engine (Caterpillar 3208, four-stroke V-8) produced a marginally positive response. Given the sample amounts available, exhaust organics from the three light-duty engines were tested using only strain TA1538. In this study, in which fuel comparison was a primary component, results for even a single vehicle (using different fuels) could vary greater than 100 times. Qualitatively, the results from other studies [Claxton, 1980, 1981a; Claxton and Kohan, 1981; Löfroth, 1981; Dukovitch et al, 1981; Dietzman et al, 1981] are in agreement with the report of Huisingh et al [1978].

The results of Claxton and Kohan [1981] demonstrating the effects of three different sample parameters are given in Table I. The three comparisons were made between (1) different runs with the same diesel engine; (2) gasoline vehicles of the same make, model, and configuration; and (3) different makes of diesel vehicle. The coefficients of variation for the revertants per mile for these three cases were 0.11, 0.49, and 0.59, respectively. Assuming normal distribution and that the coefficient of variation was, in this case, a good estimation of the true standard deviation, one can estimate confidence limits in all three cases. For the above three cases, a value could fall within 99% confidence limit values and vary by 33%, 147%, and 177%, respectively. If multiple testing facilities, fuels, and bioassay laboratories were used, the variation between results would be expected to increase. Because the Ames assay is a semiquantitative test for screening substances over a dynamic range of $\sim 10^6$ in a dose-response slope and because other parameters (such as percent of the particles extractable) show broad variation, the variation encountered here for a complex

TABLE I. Comparison of Summary Data Demonstrating the Effect of Differing Sampling Parameters

	Slope[a] rev/plate/μg	% Extractable	Rev × 10^5 g particle	PER[b] g/mi	Rev × 10^5/mi
Different runs within same automobile (diesel)					
Mean	3.68	11.8	4.35	0.524	2.27
Standard deviation	0.42	1.0	0.64	0.037	0.26
Coefficient of variation	0.11	0.09	0.15	0.07	0.11
Vehicles of same make, model, and configuration (gasoline)					
Mean	7.03	7.52	3.16	0.0102	0.032
Standard deviation	3.51	7.83	0.87	0.0048	0.016
Coefficient of variation	0.50	1.04	0.28	0.47	0.49
Different diesel vehicles					
Mean	1.98	36.6	6.96	0.687	4.38
Standard deviation	0.80	18.0	4.06	0.256	2.59
Coefficient of variation	0.41	0.49	0.58	0.37	0.59

[a]Slope of linear regression line.
[b]Particle emission rate.

testing situation should not be considered excessive. Together, these studies indicate the degree to which quantitative comparisons can be made within a single study and show that cautious qualitative comparisons can be made using results from multiple studies.

Ambient Environmental Conditions

Ambient conditions are known to affect the condensation of organic compounds onto particles, influence the interaction of organic compounds, alter the organic species emitted by a source, and provide the conditions for various other interactions [Pitts et al, 1982a]. Dilution-tunnel experiments examining the effect of crankcase oil temperatures upon test results were reported by Braddock [1981]. After the vehicles were maintained overnight at various ambient temperatures (ranging from 23°F to 82°F), the vehicles were tested at ambient temperatures. For the soluble organic fraction, a mild correlation between mutagenic activity and conditioning temperature was noted; however, this correlation did not exist for comparisons on a revertant per mile basis. Only a few investigators have explored ambient effects. Claxton and Barnes [1981] used the Calspan smog chamber to examine a variety of ambient factors. They found that the presence of ozone in the chamber tended to reduce the mutagenic response to the organic material collected. Those results also showed that ambient like irradiation without other mitigating factors such as ozone did not alter the mutagenic response.

Ohnishi et al [1980] examined road side particles collected in a highway tunnel. They found a 60- to 88-revertants/m^3 response for particles collected during daytime hours and tested with TA100 in the presence of an activating system. In the same study, particles collected at night with a high density of diesel traffic exhibited 121 to 238 revertants/m^3. Alfheim and Moller [1981] found that the contribution of traffic to the mutagenicity of air samplers is significant by comparing samples from a roadside site, on a roof, and at a park. In an Allegheny tunnel study conducted by Pierson et al [1982], the diesel aerosol organics were similar in activity to organics recovered in dilution tunnel studies. Furthermore, it was shown that the mutagenicity of diesel engine exhaust is several times that of gasoline engine exhaust when expressed as revertants per mile. Studies such as these demonstrate that the production, chemical alteration, distribution, and concentration of mutagenic mobile source particles are dependent upon traffic patterns, amounts of reactive gases and vapors, level of ozone present, meteorological conditions, and the presence or absence of other ambient air particles.

Effect of Sample Collection Upon the Mutagenicity of Mobile Source Emissions

The influence of particle collection methods upon the chemical composition and biological activity of diesel-particle extracts was investigated by Chan et al [1981]. They noted that filter sampling allowed potential chemical conversion of organic compounds by the nitrogen oxides in the exhaust gases, whereas electrostatic precipitation (ESP) collection methods provided for ozone generation and interaction at the time of collection. In their experimental results, they found 11% extractable organics for the ESP sample and 6.2% for the filter collected sample. The chemical profiles for the two collection methods were similar except that the ESP sample contained greater amounts of an acid salt fraction. Although the overall biological activity of the ESP and filter samples was comparable, subtle but consistent differences sug-

gested that different direct-acting mutagens could be found in the two sample types. As seen from the studies of Chan et al [1981], the most serious obstacle in collection methodology is the generation of artifacts, ie, the generation of substances that do not exist in the natural situation or the elimination of substances that would normally exist. A number of investigators [Claxton, 1980; Claxton and Kohan, 1981; Löfroth, 1981; Gibson et al, 1980; Pederson and Siak, 1980, 1981a,b] have demonstrated that mutagenic nitroarenes are contained in organic extracts of filter-collected particles. However, since diesel and gasoline also emit varying levels of nitrogen oxides that pass across the filters and collected particles, these nitroarenes may be artifacts. They may be produced under three possible circumstances: (1) the combustion process; (2) the exhaust process, as organics interact and condense upon the particles; or (3) the collection process as an artifact. The passage of nitrogen oxides across a PAH compound upon a filter can generate a nitroarene [nitrogen dioxide (NO_2)-PAH] that is direct-acting in the Ames bacterial assay [Pitts, 1979].

Henderson et al [1981] generated direct-acting mutagens for strain TA100 by exposing 1-g samples of fuel aromatics and fuel aliphatics to excess NO_2 at 25°C. The aromatic NO_2 fraction was the most active and nitro-PAH compounds were identified in this fraction. In some preliminary experiments, Bradow [1980] and Claxton [1980] reported passing artificial gas streams containing high levels of NO_2 across filters with diesel particles and observing increased mutagenic activity of the extracted organics. Gibson et al [1980] reexposed filter-collected diesel particles to the gas-phase portion of similar diesel emissions and found increased levels of 1-nitropyrene, nitrobenzo(a)pyrene, and mutagenic activity. Although the issue of the extent and relevance of artifacts has not been fully resolved, bacterial testing has paved the way in identifying and providing methods for examining the problems.

Since sample collection must occur during some type of test cycle (running test modes of the vehicle or engine), the test cycle may affect the generation, transformation, condensation, and collection of emitted particles and organics. Only a few researchers have published any direct comparison of test cycles. When reporting data as revertants per microgram of organic material, Gabele et al [1981] found no great differences between six different test cycles. Gibbs et al [1980] examined five different cycles with six different automobiles. When expressing the data as revertants per gram of particulates, they found "widely divergent" results; however, when expressing the data as revertants per mile, "cycle-to-cycle" trends were more pronounced and reproducible. For cycles ranked by revertants per mile, activity decreased in the order Federal Testing Procedure (FTP) > Congested Freeway Driving Schedule (CFDS) > Highway Fuel Economy Test (HFET) > 50-Mile-an-Hour Cruise Procedure (50C), and a general reduction in revertants per mile was found as the mileage of the vehicle increased. Upon close examination of the data of Gibbs et al [1980], it was noted that very low-mileage cars (< 4,000 miles) demonstrated a greatly enhanced mutagenic response for all cycles except idle. McClellan [1980b] examined four test cycles using a single automobile and reported that the cycles with lower speeds and more stops and starts resulted in higher mutagenic activity.

INTEGRATION OF PHYSIOCHEMICAL INFORMATION AND PROCEDURES WITH BACTERIAL BIOASSAY PROCEDURES

A review of the literature prior to 1979 provided a list of 184 chemicals identified as being in diesel exhaust [Claxton, 1982]. Of these 184 compounds, 44

were listed in published mutagenicity reports and 21 were acceptable positives in one or more mutagenicity assays. Seven of the 184 were reported as carcinogens. Since 1979, more research activity has been devoted to bioassay-directed fractionation than to the pairing of chemical and biological literature reports. For showing the mutagenic response of different chemical fractions from the organic emissions of a diesel engine, an organic extract from emission particles of two heavy-duty engines was initially used [Huisingh et al, 1978]. The two most active fractions, the transitional and the oxygenate, were eluted from a silica gel column after dichloromethane (DCM) extraction from the exhaust particles. Choudhury and Doudney [1981] fractionated organic emission into three primary fractions—acid, basic, and neutral—and subsequently fractionated the neutral fraction into seven subfractions. All three major fractions and five of the seven subfractions showed some type of mutagenic activity. The paraffinic subfraction was negative.

Upon examining emissions from both a diesel and a gasoline vehicle, Löfroth [1981] noted that the aromatic and an oxygenate fraction were the most mutagenic. McClellan's work [1980a], using a Fiat under varying conditions, showed that upon Sephadex fractionation three of five fractions were mutagenic to bacteria. The classes of compounds reported as contributing to the mutagenicity of these fractions were alkyl-substituted PAH compounds and oxygenated PAH. Ohnishi et al [1980] investigated the fractionated emissions of two heavy-duty vehicles and one small diesel and found the fractions to be positive. Rappaport et al [1980] studied 16 liquid chromatography fractions of organic emissions from a Cummins turbodiesel engine and postulated that pyrene-3,4-dicarboxylic acid anhydride and similar compounds accounted for a sizeable portion of the mutagenic activity.

In summarizing their fractionation study with emission organics from a GM 5.7-liter diesel engine, Siak et al [1979] stated that "more than 90% of the biological activity was accounted for in the neutral-nonpolar II, neutral polar, weak and strong acid fractions." Using nitroreductase-deficient strains of the Salmonella tester strains, Claxton [1980a] and Löfroth [1981] demonstrated the presence of nitroarenes in diesel exhaust organics. Pederson and Siak [1981a,b], using normal-phase and reverse-phase thin layer chromatography, showed that monosubstituted nitro-PAH compounds were present in diesel exhaust extracts. A number of investigators have reported the isolation and identification of these potentially mutagenic nitro-PAH compounds. Xu et al [1982b] reported the tentative identification of more than 50 nitro-PAHs in an extract of diesel exhaust particles. Schuetzle et al [1982] reported the analytical methods and identification of specific nitro-PAHs associated with diesel particles. The report of Pitts et al [1982b] provided the quantitative level and bacterial mutagenicity of 3 nitro-PAHs [nitropyrene, 9-nitroanthracene, and 6-nitrobenzo(a)pyrene] and 5H-phenanthro (4,5-a,b,d)pyran-5-one for an exhaust particle extract from a six-cylinder, light-duty diesel engine. Yergey et al [1982] employed a unique and highly controlled model system in attempting to identify mutagens arising from diesel combustion. To simplify the combustion process, they used a single cylinder diesel engine, a defined fuel (1:1 volume ratio of n-tetradecane and 2,2,4 trimethylpentane), a synthetic ashless lubricating oil (polyalkylene glycol), and, for some experiments, an argon/oxygen oxidant system. Their results indicate two useful observations: (1) Nitropyrene was isolated and identified in each of the air oxidant samples and one of the argon/oxygen oxidant samples; and (2) the compounds observed are similar to those identified by investigators using typical diesel fuels. (Note that the nitropyrene

associated with the argon/oxygen sample was observed when the experiment was conducted with a cracked piston ring allowing entry of nitrogen-containing air to the chamber.) These results suggest that the formation of nitroarenes is not dependent upon fuel-bound or lubricant-bound nitrogen and that some of the PAH compounds are products of the combustion process.

At EPA's 1981 Diesel Emissions Symposium, Raleigh, NC, several investigators provided lists of compounds recently identified in diesel exhaust. Since a listing of these results may not be readily available, a compilation of the compounds is given in Table II. Table II also provides a summary of the bacterial mutagenicity associated with the compounds tested and reported. It is interesting to note that in bioassay-directed fractionation, very few investigators used the indicator strains TA1535 and TA1538; therefore, some mutagens that cause base pair substitution and that need activation to be frameshift mutagens could be overlooked. In any event, bioassay-directed fractionation has aided in the identification of several mutagens that previously have not been recognized in mobile source emissions.

USE AND EFFECT OF VARIOUS BIOLOGICAL AND ASSAY PROCEDURES

Since initial testing involved organic chemicals extracted from particles with strong organic solvents, researchers questioned whether chemicals bound to carbonaceous particles would be released into physiological fluids in vivo. McGrath et al [1978], using the Ames bioassay, tested whole particles suspended in dimethylsulfoxide (DMSO) and obtained results ranging from negative to moderately positive. However, DMSO is a moderately effective solvent. Siak et al [1981] reported extracting particles with four simulated biological fluids: fetal calf serum, 0.5% bovine serum albumin, lung surfactant, and saline. The assay of each biological fluid in the Ames test was negative except for a positive response with the fetal calf serum. The fetal calf serum extract provided only about 6% of the response found with extraction by DCM. Brooks et al [1980] found similar results with dog serum, lung lavage fluid, saline, dipalmitoyl lecithin, and albumin. However, they state that "the minimal mutagenic activity . . . may be due to a lack of removal of mutagens from the particles or an inactivation of removed mutagens by binding or some other process." Clark and Vigil [1980] tested a DCM diesel extract under the following conditions: Aroclor 1254-induced rat liver S9, an uninduced S9, an S9 without nicotinamide adenine dinucleotide (NAD), bovine serum albumin, and fetal calf serum. They found a decreased mutagenic response in each case. That result suggests that protein binding of mutagenic components was at least partially responsible for the lack of activity seen with incubated particles. By following the mutagenic activity of the DCM extracts in serum, lung cytosol, protease-treated serum, protease-treated lung cytosol, and extracted particles, King et al [1981] demonstrated the release of mutagens from diesel particles and postulated that the lack of mutagenic response is due to either protein binding or metabolism. Siak and Strom [1981] exposed rats to diesel particles, recovered the lung macrophages, and extracted the macrophages with DCM. They showed that although the particles continued to contain mutagens, "seven days after exposure, DCM extracts of alveolar macrophages had no detectable mutagenic activity, even though more diesel particles were recovered." These effects may be due to either protein binding or metabolism. Wang and Wei [1981] and Wang et al [1981] gave evidence that the antimutagenic effect of S9 is not enzymatic by

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
1.	Acenaphthalene	34493-60-2	Yergey et al [1981] Riley et al [1982]	Kaden et al [1979]	+ (8-Az)
2.	Acenaphthalene, nitro	—	Riley et al [1982] Xu et al [1982a]	—	—
3.	Acenaphthalene, nitromethyl	—	Riley et al [1982]	—	—
4.	Anthracene	102-12-7	Prater and Schuetzle [1982] Yergey et al [1981]	Anderson and Styles [1978]	Neg
				Epler et al [1978a, 1979]	Neg
				Florin et al [1980]	Neg
				Gibson et al [1978]	Neg
				Lavoie et al [1979]	Neg
				Probst and Hill [1980]	Neg
				Salamone et al [1979]	Neg
5.	Anthracene, methyl	—	Prater and Schuetzle [1982]	—	—
	2-methyl	613-12-7	—	Gibson et al [1978]	Neg
	9-methyl	779-02-2	—	Kaden et al [1979]	+ (8-Az)
			—	Epler et al [1978a]	?
			—	Gibson et al [1978]	Neg
			—	Kaden et al [1979]	+ (8-Az)
6.	Anthracene, dimethyl	29063-00-1	Prater and Schuetzle [1982]	Hubbard et al [1981]	+
7.	Anthracene, trimethyl	27358-28-7	Prater and Scheutzle [1982]	—	—
8.	Anthracene, tetramethyl	—	Prater and Schuetzle [1982]	—	—
9.	Anthracene, nitro	—	Riley et al [1982] Xu et al [1982a]	—	—
	9-nitro	602-60-8	—	Ho et al [1981]	+
				Matsushita [1980]	+
				Pederson and Siak [1980, 1981a]	+
				Tokiwa et al [1981]	+
	2-nitro	—	—	Claxton and Kohan [1981]	+
10.	Anthracene, nitromethyl	—	Riley et al [1982]	—	—

11.	Anthracene, nitrodimethyl	—	Riley et al [1982]	—	—
12.	Anthracene, carboxyaldehyde	—	Prater and Schuetzle [1982]	—	—
13.	Anthracene, carboxyaldehyde, nitromethyl	—	Riley et al [1982]	—	—
14.	Anthracene, benz(a)	56-55-3	Prater and Schuetzle [1982]	de Flora [1981]	+
				Glatt et al [1981]	+
				Probst et al [1981]	+
15.	Anthracene, dione, benz	—	Prater and Schuetzle [1982]	—	—
16.	9,10-anthraquinone	84-65-1	Erickson et al [1982]	Anderson and Styles [1978]	Neg
				Brown et al [1977]	Neg
				Gibson et al [1978]	Neg
				Kaden et al [1979]	Neg
				Salamone et al [1979]	Neg
17.	Anthraquinone, nitro	—	Xu et al [1982a]	—	—
	1-nitro	82-34-8	—	Matsushita [1980]	+
18.	Anthrone	90-44-8	Prater and Schuetzle [1982]	Anderson and Styles [1978]	Neg
			Erickson et al [1982]	Brown et al [1977]	Neg
				Gibson et al [1978]	Neg
				Kaden et al [1979]	Neg
19.	Anthrone, nitro	—	Riley et al [1982]	—	—
20.	Anthrone, methyl	—	Prater and Schuetzle [1982]	—	—
21.	Anthrone, dimethyl	—	Prater and Schuetzle [1982]	—	—
22.	Anthrone, trimethyl	—	Prater and Schuetzle [1982]	—	—
23.	Benzo(a)pyrene	50-32-8	Prater and Schuetzle [1982]	Epler et al [1978a]	+
				Florin et al [1980]	+
				Pederson and Siak [1981a]	+
				Lavoie et al [1979]	+
				Pitts et al [1978]	+
				Pitts [1979]	+
				Salamone et al [1979]	+

Continued

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC (Continued)

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
24.	Benzo(a)pyrene, nitro	—	Riley et al [1982]	—	–
	1-nitro	—	—	Pitts [1979]	+
	3-nitro	—	—	Pitts [1979]	+
	6-nitro	63041-90-7	—	Pitts et al [1978]	+
				Tokiwa et al [1981]	+
				Wei et al [1978]	
25.	Benzo(e)pyrene, nitro	—	Riley et al [1982]	—	—
26.	Biphenyl	92-52-4	Yergey et al [1981]	Anderson and Styles [1978]	Neg
				Bronzetti et al [1981]	Neg
				Epler et al [1978a]	Neg
				Kawachi et al [1980]	Neg
				Probst and Hill [1980]	Neg
				Probst et al [1981]	Neg
27.	Biphenyl, nitro	—	Riley et al [1982] Xu et al [1982a]	—	—
	2-nitro	86-00-0	—	Anderson and Styles [1978]	+
				El-Bayoumy et al [1981]	+
				Matsushita [1980]	Neg
				McMahon et al [1979]	Neg
				Probst and Hill [1980]	Neg
				Tokiwa et al [1981]	+
	3-nitro	2113-58-8	—	El-Bayoumy et al [1981]	+
				Matsushita [1980]	Neg
				McMahon et al [1979]	Neg
	4-nitro	92-93-3	—	Anderson and Styles [1978]	+
				El-Bayoumy et al [1981]	+
				Matsushita [1980]	+
				McMahon et al [1979]	+
				Probst and Hill [1980]	+
				Probst et al [1981]	+

28.	Biphenyl, nitromethyl	—	Riley et al [1982]	—	—
			Xu et al [1982a]	—	—
	2-methyl-4-nitro	33350-73-1	—	El-Bayoumy et al [1981]	+
	3-methyl-4-nitro	69314-47-2	—	El-Bayoumy et al [1981]	+
29.	Biphenyl, dinitromethyl	—	Henderson et al [1982]	—	—
30.	Biphenylene	259-79-0	Yergey et al [1981]	—	—
31.	Chrysene	218-01-9	Yergey et al [1981]	Barfknecht et al [1981b]	
				Epler et al [1978a,1979]	+
			Prater and Schuetzle [1982]	Florin et al [1980]	+
				Gibson et al [1978]	Neg
				Lavoie et al [1979]	+
				McCoy and Rosenkranz [1980]	Neg
				Salamone et al [1979]	+
32.	Chrysene, nitromethyl	—	Yergey et al [1981]	—	—
33.	Cinnoline, benzo(c)	230-17-1	Yergey et al [1981]	—	—
34.	Dibenzo(b,d)thiophene	132-65-0	Prater and Schuetzle [1982]	Kaden et al [1979]	Neg (8-Az)
				Nakamura and Kashimoto [1979]	Neg
35.	Dibenzothiophene, methyl	30995-64-3	Prater and Schuetzle [1982]	—	—
36.	Dibenzothiophene, dimethyl	70021-47-5	Prater and Schuetzle [1982]	—	—
37.	Dibenzothiophene, tetramethyl	—	Prater and Schuetzle [1982]	—	—
38.	Dibenzothiophene, carboxyaldehyde	—	Prater and Schuetzle [1982]	—	—
39.	Fluoranthene	206-44-0	Riley et al [1982]	Barfknecht et al [1981a]	+ (8-Az)
			Yergey et al [1981]	Epler et al [1979]	?
				Florin et al [1980]	Neg
				Gatehouse [1980]	Neg
				Kaden et al [1979]	+ (8-Az)
				Lavoie et al [1979]	+
				Rao et al [1976]	+
				Salamone et al [1979]	Neg

Continued

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC (Continued)

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
40.	Fluoranthene, methyl	—	Prater and Schuetzle [1982]	—	—
	1-methyl	25889-60-5	—	Lavoie et al [1979]	+
	2-methyl	33543-31-6	—	Lavoie et al [1979]	+
	3-methyl	1706-01-0	—	Lavoie et al [1979]	+
	7-methyl	23339-05-1	—	Lavoie et al [1979]	+
	8-methyl	20485-57-8	—	Lavoie et al [1979]	+
41.	Fluoranthene, nitromethyl	—	Riley et al [1982]	—	—
42.	Fluoranthene, quinone, nitro	—	Yergey et al [1981]	—	—
43.	Fluoranthene, benzo (ghi)	203-12-3	Yergey et al [1981]	Lavoie et al [1979]	+
44.	Fluorene	86-73-7	Yergey et al [1981]	Epler et al [1978a]	Neg
				Gibson et al [1978]	Neg
				Kawachi et al [1980]	Neg
				Lavoie et al [1981a]	Neg
				Probst et al [1981]	Neg
				Probst and Hill [1980]	Neg
45.	Fluorene, nitro	—	Xu et al [1982a]	—	—
	2-nitro	609-57-8	—	Anderson and Styles [1978]	+
				McCoy et al [1981]	+
				Pederson and Siak [1981a]	+
				Probst and Hill [1980]	+
				Tokiwa et al [1981]	+
46.	Fluorene, dinitro	—	Xu et al [1982a]	—	—
	2,5-dinitro	15110-74-4	—	Matsushita [1980]	+
	2,7-dinitro	5405-53-8	—	Levin et al [1979]	+
				Matsushita [1980]	+
				McCoy et al [1981]	+
				Pederson and Siak [1981a]	+
				Probst and Hill [1980]	+
				Tokiwa et al [1981]	+

47.	Fluorene, nitromethyl	—	Xu et al [1982a]	—	—
48.	Fluorene, quinone	—	Yergey et al [1981]	—	—
49.	Fluorenone	486-25-9	Erickson et al [1982] Prater and Schuetzle [1982]	Florin et al [1980]	Neg
50.	Fluorenone, benzo	76723-60-9	Riley et al [1982]	—	—
51.	Fluorenone, nitro(s)	—	Riley et al [1982] Xu et al [1982a]	—	—
	3-nitro	42135-22-8	—	Pederson and Siak [1981a]	+
	2,7-dinitro	31511-45-8	—	Levin et al [1979]	+
				Probst and Hill [1980]	+
52.	Fluorenone, nitromethyl	—	Riley et al [1982]	—	—
53.	Furan, dibenzo	132-64-9	Yergey et al [1981]	—	—
54.	Furan, 7-methyl benzo	17059-52-8	Yergey et al [1981]	—	—
55.	Indene, nitro	—	Xu et al [1982a]	—	—
56.	Indene-1-one, dihydro	—	Yergey et al [1981]	—	—
57.	Naphthalene	91-20-3	Yergey et al [1981]	Anderson and Styles [1978]	Neg
				Florin et al [1980]	Neg
				Ho et al [1981]	Neg
				Kaden et al [1979]	Neg
				Epler et al [1979]	Neg
58.	Naphthalene, dinitromethyl	—	Henderson et al [1982]	—	—
59.	Napthalene, nitromethyl	—	Henderson et al [1982] Xu et al [1982a]	—	—
	1-nitro-2-methyl	881-03-8	—	El-Bayoumy et al [1981]	+
	1-methyl-2-nitro	63017-87-8	—	Matsushita [1980]	+
	3-methyl-2-nitro	—	—	El-Bayoumy et al [1981]	+
60.	Naphthalene, nitrodihydroxy	—	Riley et al [1982]	El-Bayoumy et al [1981]	+
61.	Naphthalene, nitrotrimethyl	—	Riley et al [1982]	—	—
62.	Naphthalene dicarboxylic acid, nitro	—	Riley et al [1982]	—	—
63.	Naphthaquinone, nitro	—	Riley et al [1982]	—	—

Continued

TABLE II. Salmonella Mutagenicity Results for Compounds Identified in Diesel Exhaust Emissions and Presented at the EPA's 1981 Diesel Emissions Symposium, Raleigh, NC (Continued)

No.	Compound	CAS No.	Reference for diesel identification[a]	Bioassay reference[b]	Bioassay result[c]
64.	Phenanthrene	85-01-8	Prater and Schuetzle [1982]	Barfknecht et al [1981b]	
			Yergey et al [1981]	Epler et al [1978a,1979]	?
				Florin et al [1980]	Neg
				Probst and Hill [1980]	Neg
65.	Phenanthrene, nitro	—	Henderson et al [1982]	—	—
			Riley et al [1982]		
66.	Phenanthrene, methyl	—	Henderson et al [1982]	—	—
			Prater and Schuetzle [1982]		
			Riley et al [1982]		
	1-methyl	832-69-9	—	Gibson et al [1978]	Neg
				Lavoie et al [1981b]	+
	2-methyl	2581-84-2	—	Gibson et al [1978]	Neg
				Lavoie et al [1981b]	Neg
	3-methyl	832-71-3	—	Lavoie et al [1981b]	Neg
	4-methyl	832-64-4	—	Lavoie et al [1981b]	Neg
	9-methyl	883-20-5	—	Gibson et al [1978]	Neg
				Lavoie et al [1981b]	+
67.	Phenanthrene-5-one, cyclopenta	—	Yergey et al [1981]	—	—
68.	Phenanthrene, quinone	—	Yergey et al [1981]	—	—
69.	Phenanthrone	—	Erickson et al [1982]	—	—
			Prater and Schuetzle [1982]		
70.	Phenanthrene, methyl	—	Prater and Schuetzle [1982]	—	—
71.	Phenanthrone, nitro	—	Riley et al [1982]	—	—
72.	Perylene	198-55-0	Prater and Schuetzle [1982]	Anderson and Styles [1978]	+
				Florin et al [1980]	+
				Ho et al [1980]	+
				Lavoie et al [1979]	+
				Salamone et al [1979]	Neg

73.	Perylene, nitro	—	Riley et al [1982]	—	—
	3-nitro	20589-63-3	—	Ho et al [1981]	+
				Pitts et al [1978]	+
				Pitts [1979]	+
74.	Pyrene	129-00-0	Prater and Schuetzle [1982]	Epler et al [1978a, 1979]	+
			Yergey et al [1981]	Florin et al [1980]	Neg
				Gibson et al [1978]	Neg
				Ho et al [1981]	Neg
				Kawachi et al [1980]	+
				Lavoie et al [1979]	Neg
				Probst ad Hill [1980]	Neg
75.	Pyrene, cyclopenta (c,d)	27208-37-3	Yergey et al [1981]	Gold and Eisenstadt [1980]	+
76.	Pyrene, cyclopenteno (c,d)	—	Barfknecht et al [1981b]	—	—
77.	Pyrene, methyl	—	Prater and Schuetzle [1982]	—	—
			Yergey et al [1981]		
	1-methyl	—	—	Kaden et al [1979]	+ (8-Az)
78.	Pyrene, nitromethyl	—	Riley et al [1982]	—	—
			Xu et al [1978a]		
79.	Pyrenequinones, nitro	—	Riley et al [1982]	—	—
80.	Thioxanthones	—	Prater and Schuetzle [1982]	—	—
81.	Triphenylenes	—	Riley et al [1982]	Epler et al [1979]	+
			Yergey et al [1981]	Gibson et al [1978]	Neg
82.	Xanthones	—	Erickson et al [1982]	—	—
			Prater and Schuetzle [1982]		

[a]All references for diesel identification are from Smith [1982].

[b]References resulting from a Environmental Mutagen Information Center (EMIC) search in September 1982. References are found within the paper's reference list.

[c]Bioassay results as reported by the authors and summarized as follows: +, positive; Neg, negative; ?, questionable or ± result; (8-Az), 8-azaquanine forward mutation system used.

examining S9, heat-deactivated S9, S9 minus cofactors, and albumin effects. Somewhat in contrast, Pederson and Siak [1981b] used a nitroreductase-deficient bacterial strain to show that some mutagens in diesel particle extracts are activated by S9 and that 1-nitropyrene is also activated by NAD phosphate-dependent S9 enzymes.

These studies demonstrate that substances are released from diesel exhaust particles into certain physiological fluids and cells. Physiological fluids and S9 apparently decrease the mutagenic activity of diesel extracts and particles primarily because of protein binding; however, some mutagenic components (eg, 1-nitropyrene) are activated by the microsomal fraction of S9, while other components are activated by the cytosol fraction.

In addition to the above concerns of scientists regarding biological parameters, investigators have questioned whether the Salmonella bioassay correlates well enough with other bioassays to use as a routine screen. This knowledge would be useful in the development of new combustion and control technologies. Lewtas showed in her review [1981] that when no exogenous activation system was used, the Salmonella bioassay data had a greater than 90% degree of correlation with the following assay data: mouse lymphoma, sister chromatid exchange in Chinese hamster ovary cells, viral enhancement, and skin tumorigenesis. When exogenous activation was used, however, the correlation with viral enhancement and skin tumorigenicity data was 79% and 72% (respectively). It will be interesting to see whether these high correlations are maintained, as the body of data and information grows.

SUMMARY

In summary, the work presented demonstrates that rapid, in vitro indicators of genotoxicity continue to play a valuable role in our understanding of the toxicity of mobile source emissions. Bacterial assays have had tremendous importance in the characterization of mobile source emissions. Specifically they have had four major uses: (1) comparative screening, (2) analyzing factors that alter the genotoxicants found in emission products, (3) directing the chemical fractionation of emission organics for the identification of specific genotoxicants, and (4) analyzing the interaction of complex emission products with various mammalian systems.

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